

ii) a probe oligonucleotide comprising a first region that is capable of specifically hybridizing to the second portion of the target polynucleotide and a flap region located adjacent to the first region; and

iii) a reagent that is capable of cleaving the flap region of the probe oligonucleotide when the probe oligonucleotide is hybridized to the second portion of the target polynucleotide and the invader oligonucleotide is hybridized to the first portion of the polynucleotide;

under conditions such that the cleaved flap region of the probe oligonucleotide and the reagent can come into contact with a reporter precursor to which the flap region of the probe oligonucleotide is capable of hybridizing to form a complex that can be, cleaved by the reagent to provide a reporter capable of being detected;

b) detecting the reporter to provide a signal; and

c) determining whether the signal exhibits a specific behavior as a function of time.

36. The method of Claim 35 wherein the invader oligonucleotide comprises a first region that is capable of specifically hybridizing to the first portion of the target polynucleotide, and a flap region located adjacent to the first region.

37. The method of Claim 36 wherein the flap region of the invader oligonucleotide is capable of specifically hybridizing to the target polynucleotide.

38. The method of Claim 36 wherein the flap region of the invader oligonucleotide is not capable of specifically hybridizing to the target polynucleotide.

39. The method of Claim 36 wherein the flap region of the invader oligonucleotide comprises a first section that is not capable of specifically hybridizing to the target polynucleotide, and a second section that is capable of specifically hybridizing to the target polynucleotide.

40. The method of Claim 35 wherein the specific behavior as a function of time is non-linear.
41. The method of Claim 40 wherein the specific behavior as a function of time is quadratic.
42. The method of Claim 35 wherein the second portion of the target polynucleotide is located immediately 3' to the first portion of the target polynucleotide.
43. The method of Claim 36 wherein the flap region of the invader oligonucleotide is located immediately 3' to the first region of the invader oligonucleotide, and the flap region of the probe is located immediately 5' to the first region of the probe.
44. The method of Claim 37 wherein the flap region of the invader oligonucleotide is located immediately 3' to the first region of the invader oligonucleotide, and the flap region of the probe is located immediately 5' to the first region of the probe.
45. The method of Claim 38 wherein the flap region of the invader oligonucleotide is located immediately 3' to the first region of the invader oligonucleotide, and the flap region of the probe is located immediately 5' to the first region of the probe.
46. The method of Claim 39 wherein the flap region of the invader oligonucleotide is located immediately 3' to the first region of the invader oligonucleotide, and the flap region of the probe is located immediately 5' to the first region of the probe.
47. The method of Claim 35 wherein the signal is fluorescence or phosphorescence.
48. The method of Claim 35 wherein the determination of whether the signal exhibits a specific behavior as a function of time is performed in real time.

49. The method of Claim 35 wherein the determination of whether the signal exhibits a specific behavior as a function of time is performed by:
measuring the value of the signal at a plurality of times to provide a data set;
fitting the data set to a polynomial function comprising a linear term and a quadratic term; and
determining whether the coefficient of the quadratic term is greater than zero.

50. The method of Claim 35 wherein the determination of whether the signal exhibits a specific behavior as a function of time is performed by:
transforming the signal to a new domain to provide a transformed signal;
fitting the transformed signal to a first mathematical function; and
comparing the shape or behavior of the first mathematical function to the shape or behavior of a linear function.

51. The method of Claim 35 wherein the second portion of the target polynucleotide is located immediately 5' to the first portion of the target polynucleotide.

52. A method comprising:

a) providing:

i) a first complex comprising a first nucleic acid, a second nucleic acid and a third nucleic acid, said first nucleic acid comprising a first portion and said second nucleic acid comprising a first region and a second region, wherein said first region of said second nucleic acid is 5' of said second region of said second nucleic acid, and wherein said first portion of said first nucleic acid is hybridized to said first region of said second nucleic acid, and wherein at least a portion of said third nucleic acid is hybridized to said second region of said second nucleic acid

ii) a fourth nucleic acid comprising a hairpin structure;

b) cleaving said first complex to generate a first cleavage product, wherein the generation of said first cleavage product exhibits a first specific behavior as a function of time;

c) hybridizing at least a portion of said first cleavage product to at least a portion of said fourth nucleic acid to form a second complex;
d) cleaving said second complex; and
e) detecting said cleaving of said second complex to generate a detectable signal, wherein the generation of said detectable signal exhibits a second specific behavior as a function of time.

53. The method of Claim 52, wherein said first specific behavior with respect to time and said second specific behavior with respect to time are different.

54. The method of Claim 53, wherein said first specific behavior with respect to time is linear.

55. The method of Claim 53, wherein said second specific behavior with respect to time is exponential.

56. The method of Claim 52, wherein said cleaving of said second complex cleaves said fourth nucleic acid.

57. The method of Claim 56, wherein said cleaving of said fourth nucleic acid is within said hairpin structure.

58. The method of Claim 52, wherein said detecting said cleaving of said second complex comprises detection of fluorescence.

59. The method of Claim 58, wherein said second complex comprises a fluorophore having quenched emission, and wherein said detecting said cleaving of said second complex comprises detection of a change in fluorescence as a function of time.

60. The method of Claim 58, wherein said change in fluorescence as a function of time is a change in intensity.

61. The method of Claim 60, wherein said change in fluorescence intensity is an increase in intensity.

REMARKS

Claims 1-34 were in the Application as filed. Claims 1-34 have been cancelled without prejudice in order to further the Applicants' business interests and the prosecution of the present Application. Applicants reserve the right to prosecute the original claims (or similar claims) in the future. Support for the new claims is found throughout the Application. One example of support for Claims 35-61 is found on page 113, lines 1-18. Support for a variety of means of detection are found throughout, including, for example, on lines 3-22 of page 13.

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